July 1, 2014

Repeated ER Intein DEAD PCR reaction with correct primers

Table 1. Protocol for Single PCR Reaction to Clone ER Intein into T7 RNA Polymerase

Reagent	**Volume (µl)**
Water	31
5X HF PCR Buffer	10
dNTPs	1
DNA (T7 N-term with overhang)	2.5
DNA (ER Intein with overhang)	2.5
DNA (T7 C-term with overhang)	2.5
Phusion Taq enzyme	0.5
	Total: 50

Table 2. PCR Conditions to Clone ER Intein into T7 RNA Polymerase

Temperature (°C)	**Time**
98	2 minutes
98	10 seconds
60	15 seconds
72	4 minutes
72	5 minute
4	Hold

- Run 2 µl of product in 1 % agarose gel
    - Bands at expected product size - weren't the main amplified product
    - Indication of binding at other locations

Table 3. Protocol for Single PCR Reaction to Clone ER Intein into T7 RNA Polymerase with outside primers

Reagent	**Volume (µl)**
Water	31
5X HF PCR Buffer	10
dNTPs	1
Primer 1 (T7XbaF)	2.5
Primer 2 (T7PstR)	2.5
DNA (PCR Reaction from Table 1.)	2.5
Phusion Taq enzyme	0.5
	Total: 50

- Run 2 µl of product in 1 % agarose gel
    - Bands at expected product size - weren't the main amplified product
    - Indication of binding at other locations

July 2, 2014

Repeated PCR to synthesize T7 RNA Polymerase + ER Intein in two parts

Table 1. Protocol for Single PCR Reaction to Synthesize T7 RNA Polymerase N-Terminus and ER Intein

Reagent	**Volume (µl)**
Water	31
5X HF PCR Buffer	10
dNTPs	1
Primer 1 (T7XbaF)	2.5
Primer 2 (OvERintR)	2.5
DNA (T7 N-Term)	1.25
DNA (ER Intein)	1.25
Phusion Taq enzyme	0.5
	Total: 50

- Repeat reaction using DNA diluted 1/10

Table 2. Protocol for Single PCR Reaction to Synthesize T7 RNA Polymerase C-Terminus and ER Intein

Reagent	**Volume (µl)**
Water	31
5X HF PCR Buffer	10
dNTPs	1
Primer 1 (OvERintF)	2.5
Primer 2 (T7PstR)	2.5
DNA (T7 C-Term)	1.0
DNA (ER Intein)	1.5
Phusion Taq enzyme	0.5
	Total: 50

Table 3. Protocol for Single PCR Reaction to Synthesize T7 RNA Polymerase N-Terminus and ER Intein DEAD

Reagent	**Volume (µl)**
Water	31
5X HF PCR Buffer	10
dNTPs	1
Primer 1 (T7XbaF)	2.5
Primer 2 (OvERintR)	2.5
DNA (T7 N-Term)	1.0
DNA (ER Intein DEAD)	1.75
Phusion Taq enzyme	0.5
	Total: 50

- Repeat reaction using DNA diluted 1/10

Table 4. Protocol for Single PCR Reaction to Synthesize T7 RNA Polymerase C-Terminus and ER Intein DEAD

Reagent	**Volume (µl)**
Water	31
5X HF PCR Buffer	10
dNTPs	1
Primer 1 (OvERintF)	2.5
Primer 2 (T7PstR)	2.5
DNA (T7 C-Term)	0.75
DNA (ER Intein DEAD)	1.75
Phusion Taq enzyme	0.5
	Total: 50

- Run in 1 % agarose gel (Gel Photo)
- Gel purify product

Table 5. Protocol for Single PCR Reaction to Synthesize T7 RNA Polymerase with ER Intein

Reagent	**Volume (µl)**
Water	31
5X HF PCR Buffer	10
dNTPs	1
Primer 1 (T7XbaF)	2.5
Primer 2 (T7PstR)	2.5
DNA (T7 N-Term + ER Intein)	2.0
DNA (T7 C-Term)	0.5
Phusion Taq enzyme	0.5
	Total: 50

Table 6. Protocol for Single PCR Reaction to Synthesize T7 RNA Polymerase with ER Intein

Reagent	**Volume (µl)**
Water	31
5X HF PCR Buffer	10
dNTPs	1
Primer 1 (T7XbaF)	2.5
Primer 2 (T7PstR)	2.5
DNA (T7 C-Term + ER Intein)	2.0
DNA (T7 N-Term)	0.5
Phusion Taq enzyme	0.5
	Total: 50

Table 7. Protocol for Single PCR Reaction to Synthesize T7 RNA Polymerase with ER Intein DEAD

Reagent	**Volume (µl)**
Water	31
5X HF PCR Buffer	10
dNTPs	1
Primer 1 (OvERintF)	2.5
Primer 2 (T7PstR)	2.5
DNA (T7 N-Term + ER Intein DEAD)	2.0
DNA (T7 C-Term)	0.5
Phusion Taq enzyme	0.5
	Total: 50

Table 8. Protocol for Single PCR Reaction to Synthesize T7 RNA Polymerase with ER Intein DEAD

Reagent	**Volume (µl)**
Water	31
5X HF PCR Buffer	10
dNTPs	1
Primer 1 (OvERintF)	2.5
Primer 2 (T7PstR)	2.5
DNA (T7 N-Term + ER Intein)	2.0
DNA (T7 C-Term)	0.5
Phusion Taq enzyme	0.5
	Total: 50

- PCR conditions same as in Table 5. June 30, 2014
- Run in 1 % agarose gel (Gel Photo)
- Gel purify product

Table 9. Protocol for Single PCR Reaction to Synthesize T7 RNA Polymerase with ER Intein

Reagent	**Volume (µl)**
Water	31
5X HF PCR Buffer	10
dNTPs	1
Primer 1 (OvERintF)	2.5
Primer 2 (T7PstR)	2.5
DNA (T7 N-Term)	0.9
DNA (ER Intein)	0.9
DNA (T7 C-Term)	0.7
Phusion Taq enzyme	0.5
	Total: 50

Table 10. Protocol for Single PCR Reaction to Synthesize T7 RNA Polymerase with ER Intein DEAD

Reagent	**Volume (µl)**
Water	31
5X HF PCR Buffer	10
dNTPs	1
Primer 1 (OvERintF)	2.5
Primer 2 (T7PstR)	2.5
DNA (T7 N-Term)	0.7
DNA (ER Intein DEAD)	1.1
DNA (T7 C-Term)	0.7
Phusion Taq enzyme	0.5
	Total: 50

- PCR conditions same as in Table 5. June 30, 2014
- Run in 1 % agarose gel (Gel Photo)
- Gel purify product

July 3, 2014

Results from yesterday's PCR: no product

Table 1. Protocol for Single PCR Reaction to Synthesize T7 RNA Polymerase N-terminus with ER Intein (half)

Reagent	**Volume (µl)**
Water	31
5X HF PCR Buffer	10
dNTPs	1
Primer 1 (T7XbaF)	2.5
Primer 2 (NcoOvR)	2.5
DNA (T7 N-Term)	1.25
DNA (ER Intein)	1.25
Phusion Taq enzyme	0.5
	Total: 50

Table 2. Protocol for Single PCR Reaction to Synthesize T7 RNA Polymerase C-Terminus with ER Intein (half)

Reagent	**Volume (µl)**
Water	31
5X HF PCR Buffer	10
dNTPs	1
Primer 1 (NcoOvF)	2.5
Primer 2 (PstT7R)	2.5
DNA (T7 C-Term)	1
DNA (ER Intein)	1.5
Phusion Taq enzyme	0.5
	Total: 50

Table 3. Protocol for Single PCR Reaction to Synthesize T7 RNA Polymerase N-terminus with ER Intein DEAD (half)

Reagent	**Volume (µl)**
Water	31
5X HF PCR Buffer	10
dNTPs	1
Primer 1 (T7XbaF)	2.5
Primer 2 (NcoOvR)	2.5
DNA (T7 N-Term)	1.25
DNA (ER Intein DEAD)	1.25
Phusion Taq enzyme	0.5
	Total: 50

Table 4. Protocol for Single PCR Reaction to Synthesize T7 RNA Polymerase C-Terminus with ER Intein DEAD (half)

Reagent	**Volume (µl)**
Water	31
5X HF PCR Buffer	10
dNTPs	1
Primer 1 (NcoOvF)	2.5
Primer 2 (PstT7R)	2.5
DNA (T7 C-Term)	0.75
DNA (ER Intein DEAD)	1.75
Phusion Taq enzyme	0.5
	Total: 50

Table 5. PCR Conditions to Clone ER Intein into T7 RNA Polymerase

Temperature (°C)	Time
98	2 minutes
98	10 seconds
60	15 seconds
72	2.5 minutes
72	5 minutes
4	Hold

- Repeat cycle 35 times
- Run 2 µl in 1 % agarose gel
- Gel purify product after each reaction before proceeding with next PCR

Table 6. Ligation Reaction of T7 RNA Polymerase + ER Intein into BioBrick Vector

Reagent	Amount (µl)
Vector DNA	2
T7 N-terminus DNA	3
T7 C-terminus DNA	3
Ligation Buffer (Thermo L)	1
Ligase Enzyme (Ligase)	1
Total Volume	10

- Ligate for 10 minutes at room temperature
- Put on ice until ready for transformation

Protocol: http://www.thermoscientificbio.com/uploadedfiles/resources/el001-product-information.pdf

Transformation of T7 RNA Polymerase + ER Intein into MACH Cells

Purpose: To clone T7 RNA Polymerase + ER Intein

- 50 µl of competent MACH cells into each ligation reaction
- Plate 400 µl transformants on LB + CAM plates
- Incubate at 37 °C overnight

iGEM Transformation Protocol: http://parts.igem.org/Help:Protocols/Transformation